

CLAIMS

What is claimed is:

- 5 1. A method of screening for a compound which modulates
phospholipase D (PLD) activity, comprising the steps of:
- a) combining a compound with phospholipase D (PLD),
thereby forming a mixture;
- 10 b) treating said mixture with phosphatidylcholine, such that a
cleavage reaction between phosphatidylcholine and PLD can occur, resulting in
generation of phosphatidic acid and choline ; and
- c) evaluating the amount of choline produced, such that a
compound which modulates PLD activity is determined.
- 15 2. The method of claim 1, wherein said compound inhibits PLD
from cleaving phosphatidylcholine.
3. The method of claim 1, wherein said compound increases
- 20 cleavage of phosphatidylcholine by PLD.
4. The method of claim 1, wherein step c) includes an oxidation step
of choline.
- 25 5. The method of claim 4, wherein said oxidation step includes
peroxidase, 4-aminoantipyrine, phenol and choline oxidase.
6. The method of claim 1, wherein said compound is PSDP.
- 30 7. The method of claim 1, wherein said compound is a PSDP analog.
8. A method of screening for a compound which modulates
intracellular signaling, comprising the steps of:
- 35 a) combining a compound with phospholipase D (PLD),
thereby forming a mixture;

b) treating said mixture with phosphatidylcholine, such that a cleavage reaction between phosphatidylcholine and PLD occurs, resulting in generation of phosphatidic acid and choline ; and

5 c) evaluating the amount of choline produced, such that a compound which modulates intracellular signaling is determined.

9. The method of claim 8, wherein said compound inhibits PLD from cleaving phosphatidylcholine.

10 10. The method of claim 8, wherein said compound increases cleavage of phosphatidylcholine by PLD.

11. The method of claim 8, wherein step c) includes an oxidation step of choline.

15 12. The method of claim 11, wherein said oxidation step includes peroxidase, 4-aminoantipyrine, phenol and choline oxidase.

13. The method of claim 8, wherein said compound is PSDP.

20 14. The method of claim 8, wherein said compound is a PSDP analog.

15. A method of screening for a compound which associates with protein phosphate-sensing domains, comprising the steps of:

25 a) contacting a Gst-Grb2 fusion protein complexed to a support with a labeled lipid compound with; and

b) evaluating the amount of labeled compound associated with said protein.

30 16. The method of claim 15, further comprising the step of treating said labeled lipid compound associated with a competing compound and evaluating the amount of labeled compound removed from said Gst-Grb2 fusion protein.

35 17. The method of claim 15, wherein said support is agarose.

18. The method of claim 15, wherein said labeled lipid compound is radiolabeled.

19. The method of claim 18, wherein said radiolabeled compound is radiolabeled PSDP or a radiolabeled PSDP analog.

20. The method of claim 16, wherein said competing compound is unlabeled PSDP, a PSDP analog, or delipidated albumin.

21. A method of screening for a compound which modulates the production of inositol trisphosphate, comprising the steps of:

a) treating polymorphonuclear leukocytes with a stimulation agent, causing activation of cell, thereby producing inositol trisphosphate;

b) treating said activated cells with a modulating compound;

and

c) measuring the effect of said modulating compound on production of said inositol trisphosphate.

22. The method of claim 21, wherein said stimulation agent is fMLP, a leukotriene, a cytokine, gm-csf, a lipopolysaccharid or CFA.

23. The method of claim 21, wherein said modulating compound is PSDP.

24. The method of claim 21, wherein said modulating compound is a PSDP analog.

25. The method of claim 21, further including the step of contacting a radiolabel to said inositol trisphosphate of step c).

26. The method of claim 21, wherein production of phospholipase C activity is determined.

27. A method of screening for a compound which modulates neutrophil activation, comprising the steps of:

- a) treating neutrophils with a stimulation agent, causing activation of said neutrophils, thereby producing inositol phosphate;
- b) treating said activated neutrophils with a modulating compound; and
- 5 c) measuring the effect of said modulating compound on production of said inositol phosphate.

28. The method of claim 27, wherein said stimulation agent is fMLP, a leukotriene, a cytokine, gm-csf, a lipopolysaccharid or CFA.

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29. The method of claim 27, wherein said modulating compound is PSDP.

30. The method of claim 27, wherein said modulating compound is a PSDP analog.

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31. The method of claim 27, further including the step of contacting a radiolabel to said inositol phosphate of step c).

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32. The method of claim 27, wherein production of phospholipase C activity is determined.

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